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Determination of the presence of *Escherichia coli* in the peel and edible part of the banana and evaluation of its growth during post-harvest process and storage at controlled temperature.

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Determinación de la presencia de *Escherichia coli* en la cáscara y parte comestible del banano y evaluación de su crecimiento durante el proceso de postcosecha y almacenamiento a temperatura controlada.

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ABSTRACT:

To ensure innocuous food for consumers, agroindustries have implemented preventive practices and have developed research that supports their processes. The banana producing guild in Guatemala required to determine the presence of *Escherichia coli* in the peel and edible part of the fruit, and to determine the growth of this bacteria during postharvest and storage processes. The study was carried out in four banana packing plants in Guatemala with two objectives; the first one was to determine if *E. coli* is able to infiltrate the edible part of the banana during postharvest washing. The second objective was to determine if there is *E. coli* in the banana peel at stowage and in simulation of controlled temperature storage. For the first objective, two boxes of banana were randomly collected from a packing line, then were transferred to the laboratory where the conditions of the washing tank were replicated and *E. coli* was introduced intentionally. The most probable number method was used to analyze the maceration of the fruit. For the second objective, five boxes of bananas were randomly collected from three packing plants. Samplings were made to the peel of two bananas per box, at stowage, and then during storage at temperatures between 17 and 18° C, during the 4th and 18th day. The results indicated absence of *E. coli* in the edible part of the fruit and in the banana peel at stowage area, and at controlled storage temperature during the evaluation time.

KEYWORDS: permeability, banana peel, banana pulp, *Escherichia coli*, washing water.

RESUMEN:

Ante la necesidad de garantizar alimentos inocuos para los consumidores, las agroindustrias han implementado prácticas preventivas y desarrollado investigación que respalde sus procesos. El gremio productor de banano en Guatemala precisó determinar la presencia de *Escherichia coli* en la cáscara y parte comestible de la fruta, y el crecimiento de esta bacteria durante la postcosecha y almacenamiento. El estudio se realizó en cuatro plantas empacadoras de banano en Guatemala con dos objetivos: el primero determinar si la bacteria E. coli logra infiltrarse hacia la parte comestible del banano durante el lavado postcosecha, y el segundo, determinar si existe presencia de *E. coli* en la cáscara del banano en estiba y en simulación de almacenamiento a temperatura controlada. Para el primer objetivo se recolectaron al azar dos cajas de banano de una empacadora, que fueron trasladadas al laboratorio donde se replicaron las condiciones de la pileta de lavado y se introdujo *E. coli* de manera intencional; posteriormente, se utilizó el método de número más probable, para analizar el macerado de la fruta. Para el segundo objetivo, se recolectaron al azar cinco cajas de banano provenientes de tres empacadoras, se realizaron muestreos en la cáscara de dos bananos por caja, tanto en estiba, como durante el almacenamiento a temperaturas entre 17 y 18 °C, los días 4 y 18. Los resultados indicaron ausencia de *E. coli* en la parte comestible de la fruta y en la cáscara del banano durante el proceso de estiba y almacenamiento en los tiempos evaluados.

PALABRAS CLAVE: permeabilidad, cáscara de banano, pulpa de banano, *Escherichia coli*, agua de lavado.

INTRODUCTION

The "White paper on Food Safety" was adopted by the European Commission in January 2000. It took Food Safety as one of its priorities. Subsequently, the Food Safety Modernization Act (FSMA) was created in 2011. It aims to update the food safety system in the United States and to prevent any disease dissemination problems. The final version of the Act was published on November 27th. 2015, coming into force in January 2016. It determined safety standards for small, medium, and big farmers both in and outside of the United States.

In Guatemala, a 95.4% banana production is exported to the United States. The 4.6% left goes to European countries and Asia (Spanish Economic and Commercial Office in Guatemala, 2016). This makes Food Safety a critical and important topic for the banana industry in Guatemala.

The new rules by the FSMA resolve an evolution in preventive procedures to guarantee safe food and free from physical, chemical, and biological contamination. Within the last classification listed, *Salmonella* spp. and *E. coli* O157:H7 microorganisms could represent a problem for banana safety. A possible biological contaminant or contamination transmitter is water, since bananas are immersed in full tanks during postharvest handling. This is done to facilitate its movement on the packaging line, to prevent ripening, and to mainly carry out the washing process (Ríos, Agudelo & Gutiérrez, 2017).

Throughout the postharvest process, fresh bananas are immersed or left floating in cleaning water. Such water, according to its nature and composition, might have pathogens or microorganisms responsible for decomposing, which in turn, could represent a high-risk pathogen infiltration through the edible part of the fruit. Rushing et al (2012), considers that "the intercellular space, found inside the product, will shrink if the warm product is dipped into cold water, as it absorbs diminutive quantities of water through the peduncle, other natural openings or separation points. This may also cause water blockage into the product through little cuts or abrasions" (p. 15). The longer the fruit remains submerged, the higher risk there is.

Infiltration tests in fresh products are very common for observation by means of dyeing, which determines how much contaminated water seeps through the fruit. In the case of bananas, the Independent Banana Growers Association (APIB, by its acronym in Spanish) evaluated the peel infiltration risk by using a strong colorant in early 2018. The purpose was, in a simple way, to visually verify the banana peel permeability. In conclusion, there wasn't any visual evidence of colorant infiltration through the peel after being exposed and submerged for two hours. Other cultivations don´t

present the same case, melon for instance, where tests are carried out more commonly and the results have demonstrated that the damage that pathogens may cause if exposed to Listeria monocytogenes in a 106 CFU/ml order, arises when reaching different mesocarp zones (Macarisin et al., 2017).

Rushing et al. (2012) has confirmed that human diseases are not associated with fresh banana intake, since "bananas are low-risk fruits and present a minimum infiltration risk due to their positive inner pressure, pushing latex from the cut-off stems" (p. 16).

In addition, investigations have been conducted on antimicrobial properties from unripe banana peel extract. They have been mainly carried out for pharmacological purposes, showing that there is an effect against *Staphylococcus aureus* and *E. coli* ATTC 25922 (ethanol extracts) (Ugoji, Adenipekun, Fagbemi, & Adelowotan, 2009); as well as Staphylococcus and Pseudomonas (aqueous extracts) (Zafar & Akter, 2011). However, a local study, whose main focus is food safety and how these fruit specific characteristics entail an advantage for its postharvest handling, still hasn't been done.

The need to prevent water-fruit cross contamination has established chlorine usage as main antimicrobial agent. Its monitoring is performed through colorimetric titration, giving parts per million as a result. Although, more precise and easy-to-search systems are nowadays required in order to monitor the suitable disinfection during the postharvest process, from washing to its conservation at a controlled temperature and refrigeration. Therefore, more packaging companies of fresh products are using sensors to determine Oxygen Potential Reduction (ORP), in order to control the state of their water disinfection systems and to standardize the parameters that validate it (Suslow, 2004). In fact, the standardized manual, designed by the Produce Safety Alliance (which appears as an implementation tool of the Food Safety Modernization Act), recommends the application of this parameters for monitoring effectiveness in water disinfectants (College of Agriculture and Life Sciences. Cornell CALS, 2017).

In regards of this, a big number of tests have been performed in 1968, in order to determine the disinfection level in water, mainly in swimming pools, making noticeable that the ORP measurement has a large amount of practical advantages to be used as parameters of proper water disinfection (Suslow, 2004). This has made possible to determine that the key indicator for microbiology quality evaluation of water is ORP and not the concentration of parts per million (ppm) of free chlorine. The ORP level recommended will be that oscillating between 650 and 750 mV (Steininger, 1985). At this level, bacteria such as *E. coli* O157:H7 and species of Salmonella can die within 30 seconds of exposure (Suslow, 2004). Therefore, the standardized manual, designed by the Produce Safety Alliance to implement the FSMA law, suggests values from 700 to 825 mV in chlorine-based disinfectants (College of Agriculture and Life Sciencies. Cornell CALS, 2017).

Between January and June 2018, tests have been performed in different banana plantation producers of the APIB (Association of Independent Banana Producers) in order to stabilize the ORP to the values mentioned before (700-825 mV). These tests have included the use of calcium hypochlorite in tablets, sodium hypochlorite solution, organic chlorine (PROVITAB 3â) in tablets, powder organic chlorine (PROVITAB 3â), paracetic acid and hydrogen peroxide. The results showed that, when using calcium hypochlorite or sodium hypochlorite to meet the ORP required, the chemical consumption would increase around seven times more than the commonly used one. For instance, the consumption of 25 to 30 calcium hypochlorite tables a day (to keep sprinklers at 2 ppm to fill the tanks) would go to 200 tablets (to keep the ORP at 650 mV in the water inside the tank). This increased the costs between 100% and 600%, depending on the chemical characteristics of water (iron, manganese, hardness). Another noticed factor has been that the use of high concentrations of sodium hypochlorite or calcium hypochlorite in water, put the fruit at a risk known as "burning", causing a

quality reduction. In the case of tests with other disinfectant agents like paracetic acid, hydrogen peroxide or organic chlorine, the daily operation costs increased in 700% to 1000% (De León et al., 2018).

Because of the increase in economic costs, an effort was made in order to establish, along with this research, a proper relation between the needs of water disinfection and the characteristics of the banana itself, through two main objectives: a) to determine if the *E. coli* bacterium is able to infiltrate the edible part of the banana during the postharvest process, specifically during the fruit washing phase using contaminated water with *E. coli* at approximately 60,000 UFC/mL. Also, b) to determine if *E. coli* is present in the banana peel at its admission to the stowage and during its transport simulation to the port under controlled temperature conditions. Therefore, this will allow to open discussion regarding the topic, mainly on low-risk crops such as the banana.

MATERIALS AND METHODS

The study methodology was created according to the established objectives, considering for both cases; a field phase and a laboratory phase.

The first phase was carried out at the packing plant SI-03, located in Santo Domingo, Suchitepéquez, Guatemala, where it was observed and registered the process by which the fruit goes through during its admission to the packing plant. Emphasis was driven towards the washing tank, since it is the place where the banana remains emerged the longest within water (between 15 and 30 min), before continuing with the packing process. The washing tanks received water with calcium hypochlorite from sprinklers at a concentration of 2 ppm, and had a product known as Laterox, whose function was to remove latex off of the fruit. It was possible to determine three important parameters within the washing tank: pH (7.5), temperature (26 °C) and residual chlorine (0 ppm), aiming to subsequently replicate the same conditions in the laboratory. Two boxes of bananas for exportation were randomly picked from the packing line (roughly 200 bananas), in order to be used in the laboratory to replicate the process and perform the corresponding exams. The boxes were wrapped in brown paper as they were transferred to the laboratory with the purpose of protecting them from external contamination. They were also storaged under room temperature the same day they got in. In the laboratory, the washing tank conditions were simulated in a square plastic container with 20 ltr of water, using the registered parameters as reference during the visit to the packing plant. Then, the water in the plastic container was contaminated with 2.0 ml of a 24-hour culture of *E. coli* ATTCC 2592 in lactic broth.

Colony-forming units per ml of contaminated water were evaluated by means of the Plate Count of Aerobic method (APHA-AWWA-WEF. Standard Methods for the Examination of Water and Wastewater. 23th ed, 2017. 9215. Heterotropic plate count). The result obtained was of 60 000 CFU/ml. Similar challenge studies to the one in mention have been practiced in order to determine the efficiency of different disinfectant agents in apples, lettuce, strawberries and cantaloupes, by using inoculums of *E. coli* O157:H7 and Listeria monocytogenes, which reach concentrations of 108 CFU/ml (Rodgers, Cash, Siddiq, & Ryser, 2004).

As criteria to define the number of samples, recommendations from the Codex Alimentarius Commission (2004), the General guidelines on sampling and the Central American Technical Regulation (RTCA, by its acronym in Spanish) 67.04.50:08 were complied. This rules establish sampling plans in order to reflect the microbiology conditions of one lot of food production, considering the concern and danger type. Bananas are in group 4, subgroup 4.1, C-risk type (food that by nature, composition, process and handling, have low probability to deteriorate health). For *E. coli*, it is established a three-type sampling plan (acceptable, moderately acceptable and unacceptable), with at least 5 units per lot (n=5) and a maximum of two sample units that can contain the analyzed microorganism (c=2), with limits of 10 CFU/g (Consejo de Ministros de Integración Económica Centroamericana, 2009).

For this study, c was reduced to 0 (c=0) and the acceptable limit was determined as absent (0 CFU/g), since it was the edible part of the fruit.

Twenty bananas were picked to be immersed into intentionally contaminated water with *E. coli* for 15 to 30 minutes. After 15 minutes, 10 bananas were taken out, the other 10 were extracted when 30 minutes were up, sterile gloves were used to do so. It was decided to take 10 samples per treatment since at least 5 units were required for the sampling plan previously described. Taking 10 samples per treatment guaranteed the duplication of the minimum quantity recommended. This quantity was fixed for convenience when considering the economic restriction of costs for each analysis in the laboratory.

One sample of all the peel from each banana was taken with a sterilized swab moistened with Neutralizing Broth, D/E Dey-Engley) Broth, found in test tubes with 10 ml each. The swab was put back into the broth to subsequently quantify *E. coli* by using the plate count method (APHA. 9.933 VRBA/ MUG Method for *E. coli* and Coliforms), in order to report on CFU/peel of each banana. After that, the sampled bananas were placed on sterile napkins and their ends were cut by using a sterile knife. The peel was first disinfected with cotton, covered in ethanol in a 70%, in order to avoid any contamination from the peel towards the edible part, which was extracted with a sterile spoon and put into a whirl pack bag for its maceration. That only sample weighed 50 g of maceration and it was analyzed following the MPN method (APHA-American Public Health Association. 9.91-9.92 *E. coli* Most Probable Number (MPN) technique).

During the second phase of the study and in order to identify *E. coli* presence, five boxes of bananas were directly picked from the packing line in the packing area, (A, B, C, D, E), coming from three different packing plants located in Caballo Blanco, Retalhuleu and Guatemala. The three packing plants reported residual chlorine concentrations in the washing tank of 0.2 ppm and in the sprinklers of 0.4 ppm. Boxes A and B were from CS-09 packing plant, box C from E01-1 packing plant, box D from E01-2 and box E from CV-07 packing plant. Boxes B, C, C and E were subjected to a usual step of the process, fungicide spraying (tiabendazole and azoxystrobin), while no spraying was applied to box A.

The five boxes were placed in the packing area, where each one was split into two hands (term referring to a group of bananas that varies from 5 to 8, attached to the stem) and two fingers (bananas) were selected, from which sampling was then taken by applying the swab technique. So, five samples were taken (one sample coming from each box) every two hours (10:18, 12:30, 14:30, 16:44 h) for a 20 samples total. The mean temperature during this sampling phase was 26°C. Then, the five boxes were storaged in a cold room with a temperature between 17°C and 18°C, since temperatures from 11°C to 12°C could cause damage due to refrigeration because of and transportation containers and ripening chambers usually function between 14°C and 18°C (Organización de las Naciones Unidas para la Agricultura y la Alimentación [FAO], 2000). For the conditioning of the boxes inside the cold room, it was considered to cover any openings in the boxes with paper in order to avoid any contamination when piling them up. On day 4 of storage, five samples were taken (one per box) at 7:54, and on day 18 of storage, five other samples were taken (one per box) at 10:50 h, for a total of thirty samples in three days (day 1= 20, day 4=5, day 18=5). All samples were transferred to the lab at a monitored temperature according to the indications by the technical personnel. From the D/E neutralizing broth, it was determined *E. coli* presence by the method APHA-American Public Health Association, 9.933 VRBA/MUG Method *E. coli* and Coliforms, where the result reports on CFU (colony-forming units) /2 bananas for *E. coli.*

The number of samples on this phase was determined for convenience (n=30) and taking into account the criteria of at least 5 units per lot (n=5) established by the Central American Technical Regulatory (RTCA, by its acronym in Spanish) 67.04.50:08 for *E. coli* surveillance on fresh fruits like bananas.

RESULTADOS

Although in some countries the use of water during postharvest process is omitted due to restricted availability of resources (Mencarelli & Mejía, 2004), in tests over the Central American region, fresh bananas are immersed in water from the washing tank, which according to its nature and composition, could contain pathogens or microorganisms, rotting agents. In turn, this could represent a bigger infiltration risk of pathogens towards the edible part of the fruit. However, after bananas being immersed in contaminated water with *E. coli* (at about 60 000 CFU/ml) for 15 to 30 min, *E. coli* presence wasn't detected in the edible part of the fruit (maceration tests) even though the pulp was extracted from fruits showing a high level of *E. coli* contamination on its peel, whose values raising from 7 400 CFU/peel on sample 7, up to 18 200 CFU/peel on sample 3 for a fruit immersion of 15 min. From 7 700 CFU/peel on sample 3 to 21 900 CFU/peel on sample 10 for a fruit immersion of 30 min, obtaining undetectable results for both conditions in the edible part, for a total 20 negative samples.

Additionally, over the second phase of the study it was determined that the 20 samples taken from banana peel didn't present any *E. coli* presence during the term of the fruit in the stowage area (1 day) and within the first hours of sampling (10:18, 12:30, 14:40, 16:44 time) at a mean temperature of 26°C. This result repeated both in the five samples taken on day 4 and the five samples taken on day 18 after the boxes were storage in the cold room at the monitored temperature between 17°C and 18°C, for a total of thirty negative samples.

DISCUSSION OF RESULTS

It´s been shown that the cross microbiologic contamination risk from the water to the inner part of bananas is low when the peel does not present any mechanical damage and also that the banana ripening state (green) does not contribute to keeping peel harshness conditions. This provides an impermeability condition that plays an important function in fruit innocuousness, mainly on its edible part, protecting it from *E. coli* contamination even 30 min after being immersed in contaminated water. It is essential to point out that a banana experiences, in average, 30 min of immersion during the washing process, more time if possible, according to Ben-Yehoshua & Mercier (2005) "Eliminating part of natural wax off of the peel, making it more susceptible. Reason why it is important to monitor both time periods of washing process and water temperature" (cited in Ramírez et al., 2011).

The impermeability condition in the banana peel occurs mainly due to its chemical composition, which has 70% of starch from dried fruits in an unripe state (Blasco & Gómez, 2014) and 80% of starch, hemicellulose and cellulose (Moreira Carrion, 2013). Specifically Wachirasiri, Julakarangka and Wanlapa (2009) indicate that banana peel has between 7% and 12% of cellulose, 6.4% and 9.6% of lignin and between a 6.4 to 8.4% of hemicellulose, which make possible to have a natural harsh peel structure that functions as a tough barrier to penetrate by microorganisms. In addition, among natural protection characteristics of the fruit, it is important to mention latex, both in the banana plant and the fruit, where "it fulfills the biological function of delaying and sometimes suppressing fungi and bacteria growth that could affect parts of the plant before reaching a physiological maturity or lose its functionality", according to Kallarackal et al. (1986) quoted from Ramírez et al. (2011). Anhwange, Ugye & Nyiatagher (2009) also mention the existence of cyanhydric acid, substance especially poisonous and oxalate in concentrations of 1.33 mg/g and 0.51 mg/g respectively (staying within the safe limits for human health (0.5-3.5 mg/g)), but probably harmless for microorganisms. It is also important to highlight that there are many researches that revealed antimicrobial

qualities from unripe banana peel extract, mainly with pharmaceutical objectives, showing that exists an action against *Staphylococcus aereus* and *E. coli* ATTC 25922 (ethanol extract) Ugoji et al. (2009). As well as against *Staphylococus aureus* (aqueous and ethanolic extract) (Romero, 2018); and Staphylococcus and Pseudomonas (aqueous extract) (Zafar & Akter, 2011).

This impermeability condition, jointly with other biochemical conditions, is very important if it is considered that water is used to protect the fruit from bumps and move it during postharvest phase, as well as for its washing process. *E. coli* presence in water is a sign of recent contamination by residual water or polluted by animal waste, which could be transmitted because of contaminated food consumption, feces contamination or through cross contamination (Rock & Rivera, 2014). There is a large number of examples of foods involved in *E. coli* O157: H7 outbreaks and a growing number of outbreaks associated with fruit and vegetable consumption (mainly collard, spinach and lettuce), contaminated by the contact with domestic or wild animal feces at some moment during its cultivation or handling (Organización Mundial de la Salud [OMS], 2018).

It was also possible to determine *E. coli* presence in the peel of a banana in the stowage area and the other that was exposed to transportation and storage simulated conditions for 18 days, where the temperature was at a rate from 17 to 18°C. The results were the same for the 30 samples on the swab technique, which were taken during the three days of sampling, even with a concentration of residual chlorine of 0.2 ppm in the tanks of the packing plants, where the fruit was selected. This number is lower than the accepted maximum limit recommended by the Regulatory Guatemalan Council (COGUANOR, by its acronym in Spanish), NTG 29 001: 2013 first review. In this regulation, it is established an acceptable, safe and desirable maximum limit of free residual chlorine of 0.5 ppm in order to reduce 99% of *E. coli* concentrations and certain viruses. Therefore, it was evident that having bananas free from *E. coli* in stowage, guarantees zero bacteria growth during storage, even though there are conditions that could develop its growth, or as indicated by Castro, Chaidez, Rubio y Valdez (2004), that the refrigeration temperature does not represent a limitation to the development and survival of these pathogen organisms once the foods are contaminated.

The Innocuousness Service and Food Survey of the United States Department of Agriculture (USDA) (2010) determines that "favorable conditions for a fast bacteria growth are those that have a temperature rate between 4.4°C and 60°C. However, other studies carried out show that even though bacteria growth may occur in proper temperature rates, growth of *E. coli* pathogen strains can be controlled when food is exposed to certain temperatures. For instance, growth stops at a temperature of 4°C for 2 to 28 days, but when temperature rises up to 20°C, it could delay for 6h or less (Jiménez García, 2016). This agrees with what the Innocuousness Service and Food Survey of the United States Department of Agriculture (2010) stated by saying that refrigeration at 4.4°C or less could protect most of foods, even stop bacteria growth.

The results obtained are important for the banana producing sector of the country, since they confirm the natural resistance that the fruit has against *E. coli* bacteria contamination going inside its inner part in whole fruit conditions (without mechanical damage); which represents a competitive advantage if compared with other crops such as cantaloupe, which tends to be more permeable.

It was possible to determine that the consumption of the banana inner part (edible pulp) wouldn´t be a route for *E. coli* contamination representing a health risk for consumers, instead, the risk for consumers would be in the contaminated peel with *E. coli*, from which the microorganism can be transferred during handling before consumption. Therefore, it is also important to "raise the handling staff knowledge through sanitary education with participative techniques and increase training of tech

supervisors during packing and storing process in order to maintain sanitary control over the risk analysis base to efficiently act towards its prevention" (Caballero & Lengomín, 1998, p. 22), making sure that the peel does not become a cross contamination route.

In conclusion, the results could show the capacity that bananas have, mainly by its peel characteristics (at a biochemical level, such as the antibacterial and antioxidant process that occurs in the unripe banana peel) (Mokbel & Hashinga, 2005) to stop the transfer of bacteria to the edible part. It holds a higher value if it is considered that historically no *E. coli* contamination cases have been reported in banana, "even though, big water ponds may be used for long time periods, infiltration risk is minimum since bananas have an inner positive pressure that pushes away the latex from the cut stems […]. Human diseases have not been associated to fresh bananas consumption" (Rushing et al. 2012, p. 16).

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